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SEQUENCE-SPECIFIC OLIGODEOXYRIBONUCLEOTIDE CLEAVAGE BY A MAJOR-GROOVE-POSITIONED METAL-BINDING LIGAND TETHERED TO C-5 OF DEOXYURIDINE

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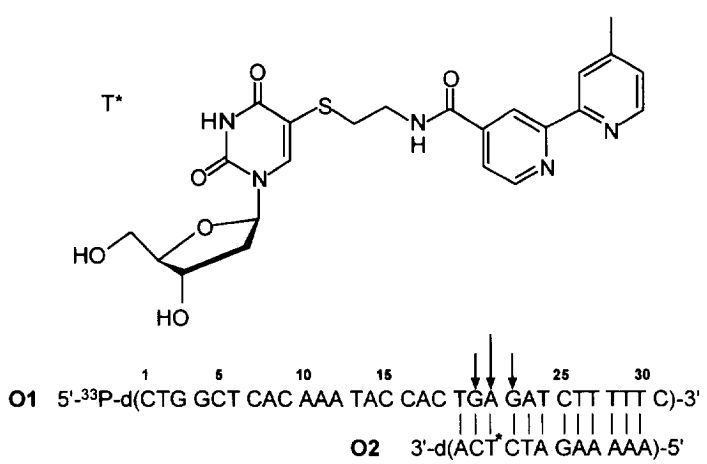
Abstract: Highly efficient cleavage of the single-stranded oligonucleotide 5'-d(CTGGCTCACAAATACCAC TGAGATCTTTTTC)-3' (**O1**) at nucleotides G20, A21, and G22 was achieved by an iron-bipyridine complex linked to a targeting oligonucleotide 5'-d(AAA AAG ATC T*CA)-3' (**O2**) through -$SCH_2CH_2NHC(O)$- at C5 of a 2'-deoxyuridine (T*). Copyright © 1996 Elsevier Science Ltd

There is substantial interest in designing chemical nucleases that are able to cleave nucleic acids with high sequence specificity and efficiency. Numerous site-specific chemical nucleases that function via metal-mediated redox or hydrolytic processes have been reported.[1-16] In an effort to increase both the specificity and efficiency of cleavage, we have been studying the effect of linking redox-metal binding ligands at nucleotide sites, which on hybridization, lead to placement of the ligand either in the minor or major groove near abstractable hydrogen atoms on the target oligonucleotide. We recently reported site-specific cleavage of a 31-mer by complementary oligonucleotides containing a phenanthroline ligand linked through N2 of deoxyguanosine. Iron-mediated abstraction of H1' and H4' deoxyribosyl protons from within the minor groove led to cleavage of the target oligonucleotide.[17]

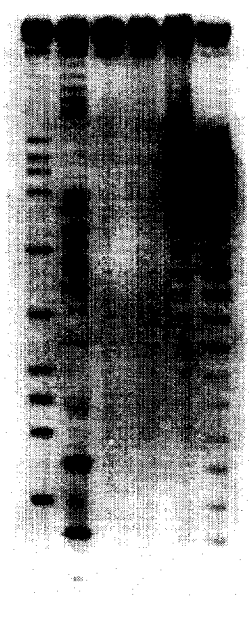
In this communication, we report the high cleavage specificity and efficiency of a single-stranded oligonucleotide (**O1,** Figure 1) mediated by a *major groove directed* iron-bipyridine complex linked to a targeting oligodeoxyribonucleotide (**O2**) through -$SCH_2CH_2NHC(O)$- at C5 of a 2'-deoxyuridine.

Figure 1



The bipyridine-conjugated oligonucleotide [**O2**, 5'-d(AAAAAGATCT*CA)-3'] is complementary to nucleotides 19-30 of **O1**. On hybridization the bipyridine would be expected to occupy the space in the major groove in the vicinity of nucleotides 19-21 (Figure 1). For redox mediated cleavage from within the major groove, the only readily abstractable atom is the deoxyribosyl H3'.[18]

Synthesis of **O2** was previously described.[19] The substrate oligonucleotide **O1** was synthesized by the standard solid-phase synthesis method with phosphoramidites as building blocks, and then purified on 20% polyacrylamide gel. 5'-End ^{33}P labeling of **O1** was accomplished with ^{33}P ATP and polynucleotide kinase from Amersham following the manufactures protocol. In our previous study we determined that the only metal ion that effectively promoted redox cleavage of a complementary sequence was Fe^{2+} in the presence of a reducing agent and oxygen.[17] This has also proven to be true in the present case. Although bipyridine complexed copper ion has been considered as a possible reagent for hydrolytic cleavage of oligoribonucleotides[20] and phenanthroline complexed copper ion is an effective reagent for the redox mediated cleavage of both DNA[2] and RNA,[21] virtually no cleavage was observed when oligonucleotides **O1** and **O2** were combined in the presence of Cu^{2+} either in the presence or absence of H_2O_2. On the other hand, in the presence of either Fe^{2+} or Fe^{3+} and H_2O_2, **O1** was cleaved in high yield. A polyacryamide gel of the products from a cleavage reaction are shown in Figure 2.



1 2 3 4 5 6

Figure 2. 0.9 pmol of ^{33}P labeled target oligo **O1** was combined with 4.5 pmol bipyridine-modified oligonucleotide **O2**, and evaporated to dryness. The mixed oligonucleotides were redissolved in 8 μL reaction buffer (50 mM TRIS pH 7.4, 50 mM NaCl) and annealed. The cleavage reaction was initiated by adding 1 μL of freshly prepared 0.1 mM ferrous ammonium sulfate and 1 μL of 4 mM hydrogen peroxide. After 30 min another 1 μL of 4 mM hydrogen peroxide was added to the reaction mixture. The reaction system was quenched after 3 hr at 22 °C by the addition of 1 μL 10 mM EDTA and 1 μL calf thymus DNA. Water was removed by evaporation and the oligonucleotides dissolved in 3.5 μL of loading buffer (formamide: 10xTBE, 9:1). The reaction mixture was denatured by heating to 90 °C for 5 min followed by cooling on ice immediately before loading onto 0.4 mm 20% polyacrylamide gel. After electrophoresis at 1800V for 3 hr, the labeled oligonucleotide was transferred to a nylon membrane by 45 min capillary blotting. The membrane was exposed to Kodak X-ray film after dryness to visualize the oligonucleotides. Lane 1: Maxam–Gilbert sequencing (A,G); lane 2: Maxam–Gilbert sequencing (C, T); lane 3: **O1**; lane 4: **O1** subjected to Fe^{2+} and H_2O_2 in the absence of **O2**; lane 5: Reaction of **O1** with Fe^{2+} and H_2O_2 in the presence of **O2**; lane 6: reaction products of O1 mediated by Fe and treated with 1M piperidine at 90 °C for 30 min.

The amount of cleavage was quantitated on a phosphorimager with Molecular Analyst® (Version 1.30). The cleavage pattern and yield is completely reproducible with the primary sites of cleavage occurring at G20,

A21, and G22. Eighty percent total cleavage was obtained with only five fold excess of **O1** (Figure 3). The three-site cleavage specificity of this study is much greater than observed for iron-EDTA mediated cleavage reactions in which hydroxyl radical is generally believed to be the responsible oxidative species. The cleavage yield is sufficiently high, that it will be of interest to see if these results can be extended to much longer sequences and to double stranded DNA. Another remarkable finding of this study is that very little self-cleavage of **O2** was observed. A control experiment in which **O2** was 5'-end labeled and subjected to Fe^{2+} and hydrogen peroxide showed less than 10% cleavage (Figure 4).

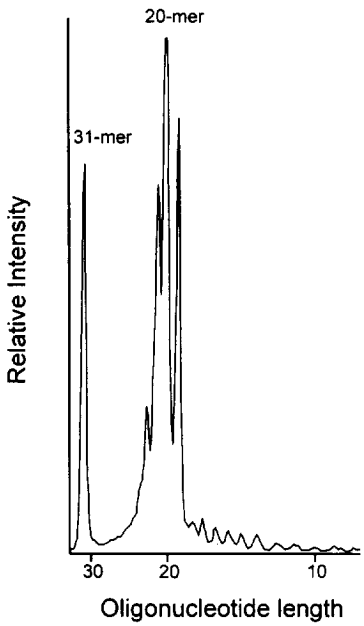


Figure 3: Phosphorimager quantitation of lane 5 (Fig. 2).

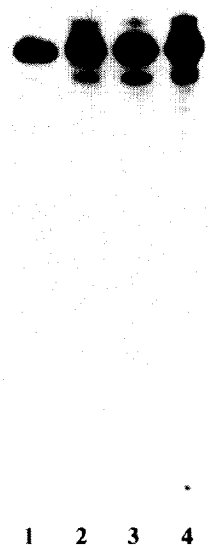


Figure 4: Lane 1: 5'-^{33}P-labeled **O2**; lane 2: **O1** (unlabeled) and **O2** in the absence of Fe^{2+} and H_2O_2; lane 3: **O2** treated with Fe^{2+} and H_2O_2; lane 4: reaction of **O2** with Fe^{2+} and H_2O_2 in the presence of **O1** (unlabeled) under the same conditions as described above in the caption to Figure 2.

The experimental results suggests that the responsible oxidative species, either metal-oxo or restricted hydroxyl radical, is highly localized around the complementary strand and the metal-ligand complex. Since no external reductant was added, it seems unlikely that a Fenton-type iron cycling process for hydroxyl radical generation is operating. If the bipyridine complexed iron is indeed localized within the major groove then the observed cleavage would be expected to result primarily through abstraction of deoxyribosyl H3'. The 5'-end labeled cleavage products co-migrated on polyacrylamide gel electrophoresis with the products of a Maxam–Gilbert sequencing reaction of **O1**. This indicates that they are terminated by a 3'-phosphate group. DNA cleavage mediated by photolysis of phenanthrenequinone diimine complexes of rhodium(III) occurs through

abstraction of a 3'-hydrogen atom to give oligonucleotides terminated primarily by 3'-phosphate.[22] In that study, lesser amounts of 3'-phosphoglycaldehyde terminated oligonucleotides apparently resulted from the reaction of dioxygen with the intermediate 3'-radical. We were not able to detect the slower moving 3'-phosphoglycaldehyde terminated oligonucleotides.

The results reported here illustrate the potential for achieving high specificity and efficiency by positioning a redox active metal–ligand complex in the major groove of an oligonucleotide duplex. The elucidation of the oxidation mechanism and further improvement of the site specificity and cleaving efficiency is actively being pursued.

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